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Advances in radioprotection through the use of combined agent regimens*

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The most effective radioprotective agents exhibit toxicities that can limit their usefulness. It may be possible to use combinations of agents with different radioprotective mechanisms of action at less toxic doses, or to reduce the toxicity of the major protective compound by adding another agent. With regard to the latter possibility, improved radioprotection and reduced lethal toxicity of the phosphorothioate WR-2721 was observed when it was administered in combination with metals (selenium, zinc or copper). The known mechanisms of action of potential radioprotective agents and varying effects of different doses and times of administration in relation to radiation exposure must be considered when using combined-agent regimens. A number of receptor-mediated protectors and other biological compounds, including endotoxin, eicosanoids and cytokines, have at least an additive effect when administered with thiol protectors. Eicosanoids and other bioactive lipids must be administered before radiation exposure, whereas some immunomodulators have activity when administered either before or after radiation exposure. For example, the cytokine interleukin-1 administered simultaneously with WR-2721 before irradiation or after irradiation enhances the radioprotective efficacy of WR-2721. The most effective single agents or combinations of protectors result in a decrement in locomotor activity, an index of behavioral toxicity. Recent evidence indicates that administration of the CNS stimulant caffeine mitigates the behavioral toxicity of an effective radioprotective dose of the phosphorothioate WR-3689 without altering its radioprotective efficacy. These examples indicate that the use of combinations of agents is a promising approach for maximizing radioprotection with minimal adverse effects.

1. Protection by thiols, antioxidants, and immunomodulators

Historically, the development of radioprotective agents has been dominated by the study of sulfhydryl compounds, particularly the aminothiols and the phosphorothioates. Various mechanisms or combinations of mechanisms for radioprotection by thiols have been proposed: at the molecular level, scavenging of reactive oxygen species, hydrogen transfer reactions, mixed disulfide hypothesis, enhancement or protection of repair enzymes, and at the biochemical-physiological level, modification of cellular metabolism, induction of hypothermia, anoxia, mediators and enzymes, and biochemical shock (Foye 1981, Weiss and Kumar 1988). The most effective thiol protectors developed thus far are S-2(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) and other

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phosphorothioates with slightly differing structures, such as S-2[(3-methylaminopropyl)amino]ethylphosphorothioic acid (WR-3689) (Davidson *et al.* 1980). A dose reduction factor (DRF) as high as 2·7 against 30-day lethality in mice has been achieved with WR-2721 (Yuhas and Storer 1969), but high levels of protection are accompanied by side-effects that may be unacceptable in many situations.

A number of 'natural antioxidants' with low toxicity, such as glutathione, superoxide dismutase, antioxidant vitamins (vitamins E, A, and C), as well as substances that mimic or induce activity of endogenous antioxidant systems (*e.g.* selenium), have been studied as radioprotectors. Generally, these natural agents provide a low degree of protection compared with phosphorothioates, but they may be of value in certain situations. Although the post-irradiation administration of antioxidants or free radical scavengers would not be expected to have much effect, evidence suggests that this may occur to some extent, and is probably related to modulation of later reactions, *e.g.* interaction of radiation-induced radicals of biomolecules with reactive oxygen species evolved during normal cellular processes (reviewed in Weiss and Kumar 1988). There is some evidence of protection against lethality for mice administered the following antioxidants after irradiation: superoxide dismutase (Petkau 1987), vitamin A (Seifter *et al.* 1984) and vitamin E (Malick *et al.* 1978). There is evidence, mostly *in vitro*, that thiols administered post-irradiation can enhance DNA repair (Riklis 1983) and reduce mutagenic effects (Grdina *et al.* 1985); however, mercaptopropionylglycine does protect against radiation-induced chromosome aberrations in bone marrow when administered to mice after irradiation (Uma Devi and Thomas 1988).

Although it is unlikely that compounds administered after irradiation will have a greater effect than those administered before irradiation that intercept or immediately repair damage or enhance repair mechanisms, from a practical point of view it is important to develop therapeutic agents, such as immunomodulators or biological response modifiers, that would enhance hematopoietic and immunological responses even when administered during the post-irradiation period.

The first and longest-studied of this class is endotoxin. When studying newer immunomodulators it is useful to recall that although endotoxin is most effective when administered 24 h before irradiation, it provides slight protection when administered shortly before or even after radiation exposure (Ainsworth 1988). An effective category of radioprotectors of the immunomodulator class is polysaccharides. The extensive studies of Patchen (reviewed in Patchen *et al.* 1988) indicate that glucan (β-1,3 polyglucose) acts as a biological response modifier when it protects against radiation exposure, e.g. when particulate glucan is given 24 h before irradiation. However, the work of Maisin *et al.* (1986) indicates that glucan and related polysaccharides may also act as free radical scavengers, because high levels of protection can be obtained by some polysaccharides when they are given shortly before irradiation (similar to aminothiols). Our studies (Weiss and Kumar 1988), using synthetic radioprotector/immunomodulators other than glucan, such as diethyldithiocarbamate (DDC) and levamisole, suggest that many immunomodulators can modulate oxidative processes and some exhibit both pro-oxidant and antioxidant properties. These anomalies point out that it is sometimes difficult to classify radioprotective agents into rigid categories. Also different mechanisms may predominate, depending on dose and time of delivery. Differences in cell biochemistry among organs or between normal and tumor tissue can result in

differential effects, including protection in one tissue and radiosensitization or toxicity in another. DDC is an example of a thiol protector with different effects (Kumar *et al.* 1986). These factors must ultimately be taken into consideration when choosing protectors of differing mechanisms for use in combinations.

2. Receptor-mediated radioprotection

Identification of specific receptors for many radioprotectors presents a great advantage as it allows a better understanding of the mechanisms of action of radioprotective agents at the cellular level. This diverse class' of radioprotective agents, having known receptors, has many subclasses and would include the bioactive lipids, naturally occurring peptides, and some synthetic compounds. Bioactive lipids that are radioprotective include metabolites of arachidonic acid, such as prostacyclin (PGI_2) and leukotriene C_4 (LTC_4); synthetic analogs of prostaglandins, such as 16,16 dimethyl prostaglandin E_2 ($diPGE_2$); and other phospholipid moieties (platelet activating factor (PAF)). The radioprotective activity of endotoxin, a lipopolysaccharide, is probably related to its lipid component (reviewed by Ainsworth 1988). The extracellular and intracellular activities thought to be involved in receptor-mediated protection are shown in figure 1.

Of the compounds acting through receptor mediation, the most active appear to be $diPGE_2$ (Hanson and Ainsworth 1985, Walden *et al.* 1987), LTC_4 (Walden *et al.* 1988), PGI_2 (Hanson 1987b), and PAF (Hughes *et al.* 1989) $DiPGE_2$ and LTC_4 effectively protect hematopoietic stem cells and intestinal crypt cells and enhance survival of irradiated mice (Hanson and Ainsworth 1985, Hanson 1987a, Walden *et al.* 1987, 1988). Of the many arachidonic acid metabolites tested, PGI_2 (Hanson 1987b) and the prostaglandin analogue misoprostol (Hanson *et al.* 1988) also provide a high degree of intestinal protection. All of these compounds are only effective when given before irradiation.

The studies of Walden and co-workers show that the maximum protection attainable by treatment with bioactive lipids (30-day survival of irradiated mice) is in the range of protection afforded by the phosphorothioates. However, when a comparison is made based on administration of equitoxic doses (1/4 LD_{10}), they are not as effective: WR-2721 or WR-3689 > $diPGE_2$ > LTC_4 = PAF. The major problems with $diPGE_2$ are extensive diarrhea and behavioral toxicity at radioprotective doses. The eicosanoids mediate many important physiological and pathological reactions, ranging from vasoregulation to inflammation, thus complicating the elucidation of the mechanisms responsible for their radioprotective properties. In addition, the eicosanoids also function as mediators of radiation injury (inhibitors of prostaglandin synthesis, such as indomethacin, can be radioprotective). Protection may involve events at the cell membrane level and induction of cell hypoxia, as well as profound physiological effects (Walden 1987).

Many biological response modifiers, such as endotoxin and glucan, induce the peptide cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF), and some of the properties of endotoxin may be due to prostaglandin and leukotriene induction as well. A common feature of synthetic immunomodulators that are protective is their ability to induce cytokines, such as colony-stimulating factors (CSF) and interleukins (Chirigos and Patchen 1988). Although the radioprotective effects of a variety of biological response modifiers may be due to enhancement of hematopoietic recovery, or other effects on a variety of immune cells, this may come about by the release of cytokines, which in turn induce the release of many

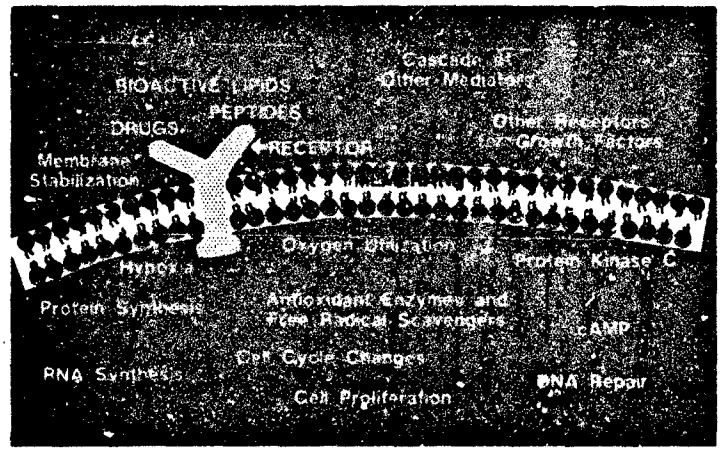

Figure 1. Cellular activities related to receptor-mediated radioprotection.

mediators, such as the products of arachidonic acid metabolism, and pathways that can be considered inflammatory (Neta 1988). Identification of IL-1 and TNF as protective and therapeutic agents against radiation (Neta 1988, Neta *et al.* 1988) presents direct evidence that inflammatory pathways participate in prevention of radiation damage and in repair, because these two cytokines are key inflammatory mediators, produced endogenously in response to multiple exogenous insults (infections, trauma and other physical stresses).

IL-1 provides varying degrees of protection, depending on the time of administration in relation to radiation exposure, probably involving a variety of mechanisms (Neta *et al.* 1988, 1989). These may include induction of hematopoietic growth factors (G-CSF, GM-CSF, IL-3 and IL-6) and induction of scavenging acute-phase proteins and superoxide dismutase, and probably other mechanisms not yet recognized (Durum *et al.* 1989). The protective effects of single doses of IL-1, whether administered before or after irradiation, contrast with G-CSF, which is effective in improving survival of mice and neutrophil recovery only when it is administered after irradiation in multiple doses (Laver *et al.* 1990). Most studies of biological response modifiers and cytokines indicate a limit of protection of 1·3 DRF.

Other peptide hormones, such as luteinizing hormone-releasing hormone (LHRH), may also protect through receptor mediation. Pre-treatment of rats with analogs of LHRH produces specific protection from radiation-induced injury to the testes (Schally *et al.* 1987). This may have important clinical applications. Methylxanthines, isoproterenol and norepinephrine act through receptors to increase cellular cyclic AMP levels, which might contribute to protective activity.

3. The problem of toxicity and combinations of radioprotective agents

A major question in chemical radioprotection remains: can protective mechanisms be separated from mechanisms of toxicity? As Maisin and Bacq (1975) have emphasized, the goal of non-toxic radioprotection appears difficult to attain because radioprotection and toxicity seem to be intimately linked in all organisms, particularly in mammals. The problem of toxicity of single protectors and combinations

is more acute when the intended use is at radiation accident sites or in space, where performance is an important factor. Behavioral and other toxicities are less problematic in clinical applications, where side-effects can be controlled. Clinical studies with WR-2721 have shown that it produces a variety of side-effects, including nausea, vomiting and hypotension (Blumberg *et al.* 1982). Animal studies indicate that WR-2721 also produces a decrement in performance (Bogo *et al.* 1985, Landauer *et al.* 1987). The use of WR-2721 as an adjunct to radiotherapy and chemotherapy should prove beneficial (Glover *et al.* 1988), especially if side-effects could be minimized. Similarly, because of exploitable differences in eicosanoid metabolism between normal and tumor tissue, the use of these bioactive lipids as protectors (or inhibition of their synthesis) in cancer treatment may some day be a reality (Walden 1987, Hanson *et al.* 1988). Protective cytokines, such as IL-1 and G-CSF, may also have considerable side-effects, but they are in clinical use.

Performance decrement must be evaluated when developing radioprotectors for uses where behavioral toxicity would be an unacceptable side-effect. In a series of studies, Landauer and co-workers demonstrated that the most effective protectors have the greatest behavioral side-effects, as measured by alterations in locomotor activity (Landauer *et al.* 1989b). In general, biological compounds produce decrements at least as large as chemical radioprotectors. Studies demonstrate that when radioprotective efficacy is compared on the basis of doses with equal behavioral toxicity, WR-2721 and WR-3689 (which have undesirable behavioral effects) are still superior to other radioprotectors tested.

There is now considerable evidence suggesting that the use of combinations of agents is a valid concept (Maisin *et al.* 1968, Patcher *et al.* 1989, Sztanyik and Santha 1976, Uma Devi and Thomas 1988, Weiss *et al.* 1987). A review of data on combined radioprotection indicates that the maximum achievable DRF, using combinations of protectors, will be approximately 3. However, it is extremely unlikely that this will prove practical because of concomitant toxicity. A more reasonable approach is to use combinations providing a lower DRF but acceptable toxicity. In addition, DRFs obtained with combined lower doses of protectors can be further extended by bone marrow transplants and other supportive care administered after irradiation. When the intended use of a combination of protectors is as an adjunct to radiotherapy, appropriate preclinical testing must be done to determine how the combination alters tumor protection versus normal tissue protection.

Throughout the remainder of this paper we discuss protection aginst γ- or X-irradiation by combinations of agents, illustrated also by previously unpublished data. In all radioprotection studies reported in the tables, CD2F1 male mice were irradiated bilaterally with cobalt-60 at 1 Gy/min. The $LD_{50/30}$ for saline-treated mice was 8·0–8·5 Gy. Other experimental details are given in Weiss *et al.* (1987) and Landauer *et al.* (1987).

4. Combinations of metals and phosphorothioates

Treatment of mice with salts of various metals (copper, zinc or selenium) provided a small radioprotective effect. Therefore, it was of interest to determine whether combinations of metals and thiol compounds would be beneficial. We first concentrated on selenium (Se) because of its known inter-relationship with endogenous antioxidant defense systems, such as vitamin E and glutathione peroxidase (Jacobs *et al.* 1983). The effect of Se, as sodium selenite, on the acute toxicity and

radioprotective effect of WR-2721, was studied in male CD2F1 mice (Weiss *et al.* 1987). Injection of 1·6 mg/kg Se 24 h before WR-2721 (800–1200 mg/kg, i.p.) decreased the lethal toxicity of WR-2721 significantly. Se injection alone (1·6 mg/kg) 24 h before cobalt-60 irradiation increased survival (DRF = 1·1). An enhancement of the protective effect of WR-2721 also occurred when Se was injected 24 h before WR-2721 (200–600 mg/kg, i.p., 0·5 h before irradiation). For example, after exposure to 22 Gy (1 Gy/min), 30-day survival was 100 per cent when mice were treated with both Se and 600 mg/kg WR-2721, and 13 per cent when they were treated with WR-2721 alone. The DRFs for 30-day survival after 400 mg/kg WR-2721 were 2·6 with Se and 2·2 without Se pre-treatment.

Pre-administration of zinc aspartate 2 min before cysteamine or 2-β-aminoethylisothiouronium-Br-HBr (AET) increased the radioprotective effect of the thiols (Floersheim and Floersheim 1986). Brown *et al.* (1988) demonstrated that zinc chloride administration 5 min before WR-2721, resulted in an increase in both the hematopoietic and gastrointestinal DRFs.

In subsequent studies we compared the effects of Se, zinc (Zn) as zinc chloride and copper (Cu) as copper sulfate. We used lower doses than those used previously in combination with thiols, and WR-2721 was also administered at a relatively low dose. Mice were either pre-treated with the metals, or the metals were administered simultaneously with WR-2721. Table 1 compares the effects of the metals on the lethal toxicity of WR-2721 (1000 mg/kg). Cu pre-administration (3 h or 24 h) resulted in an increase in survivors, but simultaneous administration with WR-2721 was not effective. Zn was effective when it was given 3 h before WR-2721. Although this study was done with a small number of animals, the results suggest that Se was more effective than the other metals when it was given simultaneously with WR-2721.

Table 2 shows that pre-treatment with each of the three metals at −3 h enhances radioprotection by WR-2721 (200 mg) at 14 Gy exposure when all treatments were i.p. However, when WR-2721 was administered orally and the metals i.p., Se was not effective, whereas Cu and Zn had a small effect. Table 3 shows the comparative radioprotective effects of solutions of metals and WR-2721 combined. In this situation, Se was the most effective, in a similar way to its effect on the lethal toxicity of WR-2721 (Table 1).

Table 1. Effect of metals (0·8 mg/kg, i.p.) on lethal toxicity of WR-2721 (1000 mg/kg, i.p., CD2F1 male mice).

Treatment	30-day survivors
WR-2721	1/30
Cu at −3 h	9/10
Cu at −24 h	7/10
WR-2721 and Cu simultaneously	1/10
Zn at −3 h	8/10
Zn at −24 h	0/10
WR-2721 and Zn simultaneously	1/10
Se at −3 h	4/10
Se at −24 h	3/10
WR-2721 and Se simultaneously	3/10

Table 2. Effect of pre-treatment with metals (0·8 mg/kg, i.p., −3 h) on radioprotection by WR-2721 in CD2F1 male mice.

	Treatment	30-day survivors
WR-2721 (700 mg/kg, p.o.), 1 h before irradiation (14 Gy cobalt-60)	WR-2721	7/48 (15%)
	+Cu	11/32 (34%)
	+Zn	18/32 (56%)
	+Se	3/32 (9%)
WR-2721 (200 mg/kg, i.p.), 30 min before irradiation (14 Gy cobalt-60)	WR-2721	15/40 (38%)
	+Cu	34/40 (85%)
	+Zn	27/40 (68%)
	+Se	34/40 (85%)

The results of metal effects on toxicity and radioprotection by WR-2721 suggest different mechanisms for the potentiation by metals of thiol radioprotection. Floersheim and Floersheim (1986) suggested that Zn stabilizes thiol protectors, but there is little experimental evidence for this mechanism. Inhibition of alkaline phosphatase by metals would alter the kinetics of conversion of WR-2721 to its active free thiol WR-1065. This might occur with higher concentrations of Zn (Brown *et al.* 1988) or Se (Weiss *et al.* 1987). The improvement in radioprotective effect of WR-2721 by Se and/or suppression of toxic metabolites formed during metabolism of WR-2721 may be due to induction of glutathione peroxidase activity by Se administration (Kumar *et al.* 1988). It is possible that other endogenous protective organometallic compounds are formed when the metals are injected, or the metals are forming new compounds by reacting with WR-1065. This appears to be most likely in the case of Se (Kumar and Weiss 1989).

Because oxygen plays an important role in the modulation of radiation sensitivity, modulation by metals of oxygen uptake by WR-1065 was investigated using an *in vitro* model system (Kumar and Weiss 1989). The highest rate of oxygen consumption by WR-1065 occurred in the presence of Cu, followed by Se, and Zn had very little effect. Purdie *et al.* (1983) suggested that WR-1065 is oxidized to the disulfide, and the consequent anoxia may contribute to protection by the drug. Our studies indicate that formation of the disulfide of WR-1065 is accelerated in the presence of increased levels of Cu. Earlier *in vivo* work by Yuhas *et al.* (1973)

Table 3. Radioprotection in CD2F1 male mice by combined treatment with WR-2721 and metals. Solutions of WR-2721 (200 mg/kg) and metals (0·8 mg/kg) administered i.p. simultaneously 30 min before irradiation.

	30-day survivors	
Treatment	14 Gy	15 Gy
WR-2721	8/16 (50%)	1/16 (6%)
WR-2721 and Cu	11/16 (69%)	9/16 (56%)
WR-2721 and Zn	13/16 (81%)	5/16 (31%)
WR-2721 and Se	16/16 (100%)	13/16 (81%)

showed that the protective effect of WR-2721 was probably influenced by oxygen tension. Denekamp *et al.* (1982) reported that an optimum level of oxygen is needed for maximum protection with WR-2721, and protection is lower below and above that level of oxygen. It is difficult to correlate our *in vitro* results with the observed radioprotective effects in mice of the metal and WR-2721 combinations, but the studies established that metal ions are important factors in the interaction between sulfhydryl compounds and oxygen with respect to radioprotection.

An important adjunct to studies on radioprotection by combinations of agents are determinations of behavioral toxicity of single agents compared with combinations of agents. Automated quantitation of spontaneous locomotor activity has been found to be a sensitive measurement of the behavioral toxicity of radioprotectors (Landauer *et al.* 1987). Table 4 summarizes the effects of combinations of metals and WR-2721 on mouse locomotor activity. Mice ($n=11$/group) were tested during the nocturnal phase of their light/dark cycle. All treatments resulted in locomotor decrements. Administration of metals alone resulted in an earlier onset of locomotor decrement than the WR-2721 treatment, but it took longer to recover from the decrement produced by WR-2721. When WR-2721 was administered with the metals, the Cu combination resulted in the most severe locomotor decrement, due mainly to the longer recovery time. The Zn and WR-2721 mixture appears to be the least toxic because WR-2721 alone produced a greater performance decrement than the combination. Information on the behavioral toxicity, lethal toxicity and radioprotective effects of combinations can be useful for comparative assessments of combined radioprotective regimens.

5. Combinations of receptor-mediated and other biological compounds with phosphorothioates

The first indication that biological response modifiers or immunomodulators might be effective in combination with thiols resulted from studies of endotoxin and AET. Administration of endotoxin at 24 h and AET 15 min before radiation exposure of mice resulted in greater than additive protection (Ainsworth *et al.* 1970). Unpublished work by Walden, using detoxified endotoxin (monophosphoryl lipid A) injected at −24 h, produced an additive effect with WR-2721 administered at −30 min.

The combined use of WR-2721 and diPGE$_2$ has been investigated (Hanson 1987a, Steel *et al.* 1988, Landauer *et al.* 1989b). The studies have shown a favorable protective response with γ-irradiation. When WR-2721 was administered 15 min before irradiation with 0·4 mg/kg diPGE$_2$ given 5 min before irradiation, 30-day survival increased as compared with that of WR-2721 alone. In this case, protection by the combined agents was slightly less than additive. The DRF for WR-2721 (200 mg/kg) was 1·9; for diPGE$_2$, 1·45; and for the combination, 2·15. A protective response that was greater than additive was obtained for survival at 6 days when diPGE$_2$ was administered 1 h before irradiation and before WR-2721 (Hanson 1987a). Greater protection of murine intestinal crypt cells was observed after the combined treatment, but protection by high doses of WR-2721 (approximately 300–400 mg/kg) could not be improved by the addition of diPGE$_2$. The greater protection produced by using the combination of agents in this study could have resulted from the order of administration, which produced different physiological responses, or from modified catabolism of the radioprotectors. Misoprostol, a synthetic analog of prostaglandin E$_1$, was effective in protecting intestinal clono-

Table 4. Behavioral toxicity (locomotor decrement) in CD2F1 male mice treated i.p. simultaneously with metals (0·8 mg/kg) and WR-2721 (200 mg/kg).

Treatment	Onset of locomotor decrement (min)	Maximum locomotor decrement		Time to recover from decrement (h)
		(min)	percentage	
WR-2721	25	25	70	4·0
Se	15	15	70	0·8
WR-2721 and Se	10	20	95	4·5
Cu	10	10	66	0·6
WR-2721 and Cu	10	30	80	7·0
Zn	10	10	35	0·3
WR-2721 and Zn	20	30	70	3·5

genic cells when combined with WR-2721 (Hanson *et al.* 1988). The combination of misoprostol (25 μg/mouse) followed by a high dose of WR-2721 (10 mg/mouse) extended the longevity of mice irradiated with 20 Gy and 23·5 Gy.

When WR-2721 followed by diPGE$_2$ was administered before fission neutron irradiation, there was no improvement in the survival of mice compared with those given WR-2721 alone (Steel *et al.* 1988). Behavioral toxicity studies conducted on mice receiving the combination of WR-2721 and diPGE$_2$ demonstrated a greater behavioral decrement measured by locomotor activity than those produced by either agent alone (Landauer *et al.* 1989b).

Although Maisin *et al.* (1986) failed to find improved radioprotection with polysaccharides and AET, Patchen *et al.* (1990) reported enhanced protection by WR-2721 in combination with glucan. The protective effect (DRF 1·2) of particulate glucan administered at −20 h was additive with the protective effect of WR-2721 (200 mg/kg, −30 min). Furthermore, an even greater protection was obtained when Se was also given at −20 h. Treatment with each of the three agents, which act by different mechanisms, resulted in an increase in hematopoietic stem cells (endogenous spleen colony-forming unit assay). Treatment with the combination of the three agents was most effective in this regard, as well as in accelerating bone marrow and splenic granulocyte–macrophage colony-forming cell regeneration (Patchen *et al.* 1990). A greater than additive effect was obtained when WR-2721 was given before irradiation and soluble glucan (Glucan-F) was administered after irradiation (Patchen *et al.* 1989). This study established a potential role for the post-irradiation use of immunomodulators in combination with traditional thiol radioprotectors. Such combinations appear to depend on the sequential thiol-mediated cell protection and immunomodulator-mediated hematopoietic stimulation.

We demonstrated that IL-1 was effective in improving the survival of irradiated CD2F1 mice when it was administered at times ranging from 20 h before to 2 h after radiation exposure. When administered after irradiation in combination with WR-2721 (200 mg/kg, −30 min), IL-1 enhanced survival at radiation doses (15–16 Gy) causing hematopoietic and gastrointestinal injury (Neta *et al.* 1989). Table 5 shows the effect of simultaneous administration of IL-1 (human recombinant interleukin-1α, Hoffmann-LaRoche) and WR-2721 at 30 min before irradiation. IL-1 alone has no protective effect at the high radiation doses tested. Greater than additive

Table 5. Radioprotective effects of combinations of WR-2721 and IL-1 in CD2F1 male mice. Simultaneous i.p. administration 30 min before irradiation.

Treatment	30-day survivors	
	14 Gy	15 Gy
WR-2721 (200 mg/kg)	14/32 (44%)	0/24 (0%)
+ IL-1 (4 μg/kg)	23/24 (96%)	6/16 (38%)
+ IL-1 (400 μg/kg)	16/16 (100%)	13/16 (81%)
	18 Gy	20 Gy
WR-2721 (400 mg/kg)	14/32 (44%)	1/32 (3%)
+ IL-1 (4 μg/kg)	30/32 (94%)	15/32 (47%)
+ IL-1 (400 μg/kg)	25/32 (78%)	6/32 (19%)

protection was obtained with combinations of IL-1 and 200 mg/kg WR-2721 (1/4 the LD_{10} dose) or 400 mg/kg WR-2721. The higher dose of IL-1 (400 mg/kg) did not provide much benefit over the lower dose (4 μg/kg). WR-2721 may be protecting hematopoietic stem cells, which in turn may be amplified because of IL-1 administration. Whether similar explanations can be used to account for improved protection with WR-2721 and IL-1 in the gastrointestinal dose range is being investigated. Although the biochemical mechanisms of protection by IL-1 are unclear, it is known that IL-1 treatment of mice induces ceruloplasmin and metallothionein (reviewed by Neta 1988), both of which have antioxidant and possible radioprotective effects. The induction of superoxide dismutase by IL-1 also was observed recently *in vitro* (Masuda *et al.* 1988). Vaishnav *et al.* (1989) showed that IL-1 can induce manganese–superoxide dismutase *in vivo*, but only at 6 h after administration with the higher dose (400 μg/kg). Therefore, superoxide dismutase may contribute to the radioprotective effect of IL-1, but may not be the only radioprotective mediator.

It would be useful to combine more than one cytokine with a phosphorothioate, because additive protection has been observed with combinations of cytokines or biological response modifiers: IL-1 and TNF (Neta *et al.* 1988), IL-1 and G-CSF (Laver *et al.* 1990) and glucan and BM41.332 (Patchen *et al.* 1988). Presently, the only recommended biological factors for the treatment of radiation injuries in humans are recombinant G-CSF and GM-CSF (Browne *et al.* 1990) because more clinical data are available for these agents. Because WR-2721 is also in clinical use, animal studies on combinations of pre-irradiation administration of phosphorothioates combined with post-irradiation administration of G-CSF or GM-CSF show promise for early acceptance for human use (Patchen and MacVittie, unpublished work). The further addition of post-irradiation bone marrow transplants has been shown previously to be of value with a number of protectors, for example, in combination with IL-1 (Oppenheim *et al.* 1989).

6. Combinations of caffeine and phosphorothioates

Research on chemical radioprotectors needs to be expanded to include studies on drugs that will prevent radiation-induced behavioral disruption and per-

Table 6. Effect of caffeine on radioprotection by WR-3689 in CD2F1 male mice. Simultaneous oral administration 1 h before irradiation.

	30-day survivors		
Treatment	10 Gy	11 Gy	12 Gy
WR-3689 (400 mg/kg)	12/32 (38%)	—	—
+caffeine (40 mg/kg)	14/32 (44%)	—	—
WR-3689 (500 mg/kg)		4/8 (50%)	2/8 (25%)
+caffeine (40 mg/kg)		10/16 (63%)	4/16 (25%)

formance decrement, as well as studies on agents that will modify the behavioral toxicity of radioprotectors (Bogo 1988). Landauer *et al.* (1989a) recently determined that the CNS stimulant caffeine can mitigate the locomotor decrement produced by WR-3689. These data and results on radioprotection by combinations of caffeine and WR-3689 will be published in detail elsewhere. The timing of caffeine administration in relation to administration of the phosphorothioate appears to be important in mitigating the behavioral effect, but not the radioprotective efficacy, of WR-3689. Caffeine administration does not have an adverse effect on the radioprotective efficacy of WR-3689 when the drugs are administered by various routes. Table 6 shows the survival of irradiated mice (10–12 Gy) after simultaneous oral administration of WR-3689 and caffeine 1 h before irradiation. No significant difference in survival was observed. Although caffeine is generally considered to be a sensitizer, results on radiosensitization effects are, in general, obtained from *in vitro* cell irradiation studies. There is evidence that, in mice, caffeine provides some protection of jejunal crypt cells, as do other inhibitors of cyclic AMP phosphodiesterase (Lehnert 1979). The results on combinations of caffeine and WR-3689 provide encouragement that the toxicities of major radioprotective compounds can be ameliorated.

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